ON THE

PHYSIOLOGICAL ACTION OF OZONE.

BY

JAMES DEWAR, Esq., LECTURER ON CHEMISTRY,

JOHN G. M'KENDRICK, M.D., Physiological laboratory, university of edinburgh.

AND

From the Proceedings of the Royal Society of Edinburgh, Session 1873-74.

EDINBURGH: PRINTED BY NEILL AND COMPANY. MDCCCLXXIV.



ON THE

PHYSIOLOGICAL ACTION OF OZONE.

A SYSTEMATIC investigation into the physiological action of ozone, so far as we are aware, has never been undertaken. Isolated observations have been made by many while engaged in the examination of its physical and chemical properties, which have chiefly tended to show that it acts as an irritant on the mucous membrane of the respiratory tract, and they have also observed the peculiar odour which it excites by its effect on the organ of smell, from which the name ozone originated. Beyond this little has been attempted. Schönbein, indeed, showed * that a mouse imprisoned in an atmosphere of ozone died in about five minutes. From meteorological data, this observer also stated that the quantity of ozone in the atmosphere and the prevalence of epidemic diseases were in an inverse relation to each other both as to time and locality. This statement has probably given rise to the popular opinion that ozone not only acts as a powerful oxidising agent of decaying animal or vegetable matters, but also that it has a specific action on the animal body.

With the view of determining what action ozone exerts on the body, we commenced a series of experimental observations, which we now beg to lay before the Society.

* British Association Reports, 1848.

1. Mode of producing the Ozone (see fig.).—The ozone in the following experiments was made by passing a current of dry air or oxygen from a gasometer (b) through a narrow glass tube, bent for convenience like the letter U (d), about 3 feet in length, and containing a platinum wire 2 feet in length, which had been inserted into the interior of the tube, and one end (e) of which communicated with the outside through the wall of the tube. Round the whole external surface of this U-shaped tube a spiral of copper wire was coiled, and the induction current from a coil giving $\frac{1}{2}$ -inch



Description of Figure. -a, glass chamber for reception of the animal; b, gasometer; the current of air or gas passed from right to left of diagram; c (to the right), bulb-tube containing sulphuric acid; c (to the left), bulb-tube containing caustic potash or water; d, U tube; e, wire from — pole of induction coil continuous with platinum wire within the U tube; f, wire from + pole of induction coil continuous with copper wire coiled round U tube.

sparks was passed between the external copper (f) to the internal platinum wire (e), so as to have the platinum wire in the interior of the tube as the negative pole. After the current of gas was ozonised by the passage of the induction current, it was washed by passing through a bulb-tube (c to the left of the U tube) containing caustic potash when air was employed, or water when pure oxygen was used, in order to eliminate any traces of nitrous and nitric acids. To the right of the U tube another bulb-tube (c) was placed containing pure sulphuric acid, for removing aqueous vapour from the air, or gas passed through it. By means of the gasometer, the volume of gas passing through the apparatus could be ascertained.

2. Method of Experiment.—It was necessary, in the first place, to determine the action of ozone on the living animal imprisoned in an atmosphere containing a large proportion of ozone; and, in the second, to determine what action, if any, it exerted on the individual living tissues of the body.

Observations were made on frogs, birds, mice, rabbits, and on ourselves.

Frogs.-Numerous experiments were made on frogs, and the general effect on these animals is as follows :- About thirty seconds after introducing the animal into the chamber, through which a steady current of ozonised air was passing, the animal manifested symptoms of distress. The eyeballs were retracted, so as to be deeply sunk in the orbits, and the eyelids were firmly closed. It rubbed its nose occasionally with its fore paws. At first somewhat restless, the frog became lethargic, and the movements of respiration were reduced, both in frequency and force, to at least onehalf the normal amount. On pushing the frog with a wire it might be excited to move, but usually it remained motionless. The position of the animal was peculiar-the neck arched, the head flattened, and it remained in a crouching attitude. This condition of lethargy has been observed to continue during a period of an hour and a half, at the end of which time the animal died. When common air was introduced into the chamber instead of ozonised air, or if the frog was taken out of the chamber, it quickly recovered. These effects may be seen in the following experiment :---

A large healthy frog was introduced into the air-chamber, through which a current of air was passing sufficient to fill a litre flask in three minutes. At the end of two minutes, the respirations were 96 per minute. The induction machine was then set to work, so as to mix ozone with the air, the current passing through the chamber at the same rate. In half a minute the eyes were affected, and the respirations were reduced to 8 per minute. At the end of six minutes, the animal was quite motionless, and the respiratory movements had entirely ceased. Pure air was then introduced. In half a minute, there was a slight respiratory movement, and in eight minutes, the respirations numbered 85 per minute. At the end of other twelve minutes, ozone was again turned on, with the same results. The animal in this experiment was then subjected to atmospheres of common air and air mixed with ozone alternately, each period of immersion in the atmosphere consisting of ten minutes, with invariably the same effect. At the end of two hours it was removed from the chamber, and recovered.

In the case of the frog which died after being exposed to an atmosphere of ozonised air for an hour and a half, the heart was found pulsating after systemic death. It was full of dark-coloured blood. The lungs were slightly congested. In every part of the body the blood was in a venous condition.

In two experiments, frogs were exposed to the action, not of air mixed with ozone, but to a stream of oxygen mixed with ozone, and the results were somewhat different from those just narrated. The effects were not so well marked. When a frog was introduced into an atmosphere of pure oxygen, the animal was lively and vivacious, the eyes were wide open, and the respiratory movements were greatly accelerated. But when the oxygen contained a considerable quantity of ozone, the eyes were closed, the respiratory movements did not entirely cease, but were reduced from 100 or 110 to 8 or 12 per minute, and the creature was in a dormant condition. After exposure for a period of one hour, the web and the skin assumed a purple hue. After keeping the animal in such an atmosphere for $1\frac{3}{4}$ hour, it was in the same condition.

Birds.—A green linnet was put into the chamber, supplied with a strong current of air. At the end of five minutes, after the bird had become quiet, the respirations were 50 per minute. The air was then ozonised. In thirty seconds, the eyes were closed; in one minute, the respirations were reduced to 30 per minute; four minutes thereafter, the respiration was slow and gasping, and the number of movements 15 per minute; and in ten minutes, that is, fifteen and a half minutes after the introduction of ozonised air, the bird was dead. On opening the body, there was venous congestion of all the viscera. The lungs were of a dark purple colour, and showed a mottled appearance. The heart was still pulsating feebly. It was full of venous blood. The brain was pale. The blood corpuscles, when examined microscopically, were normal.

Mammals.-Several experiments were made on white mice and rabbits. With regard to mice, the general effects will be understood by detailing one experiment. A full-grown and apparently healthy white mouse was introduced into a vessel through which a stream of air was passing at the rate of 8 cubic inches per minute. Five minutes thereafter, the animal was evidently at ease, and the respirations were 136 per minute. The air was then ozonised. One minute after, the respirations were somewhat slower, but could not be readily counted, owing to the animal moving uneasily about and rubbing its nose with its fore paws. In four minutes from the time of introduction of the ozone, the respirations were 32 in a minute. The mouse now rested quietly, occasionally yawned, and when touched by a wire, moved, but always in such a direction as to place its head away as far as possible from the stream of ozonised air. At the end of fifteen minutes, the animal became excited, ran rapidly backwards and forwards, and then had a convulsive attack. It died, much convulsed, nineteen minutes after the introduction of the ozone. The body was colder than natural. There was venous congestion of all the abdominal viscera. The heart was still feebly pulsating, and the right auricle and ventricle were full of venous blood. The left side of the heart contained a small quantity of venous blood. The sinuses of the brain were full of dark blood, and the surface and base of the brain was traversed by vessels containing dark-coloured blood.

Two experiments were also made upon mice, in which, instead of being supplied with ozonised air, they received ozonised oxygen. When a mouse breathed an atmosphere of pure oxygen, it became exceedingly active in its movements. It ran about examining every part of its prison, and breathed with such rapidity as to make it impossible to count the number of respirations taken during a minute. When the oxygen was ozonised, the mouse quickly showed the usual phenomena of the closed eyes and the reduction of the number of respirations, but it lived for a much longer period than in ozonised air. Instead of dying at the end of fifteen or twenty minutes after the introduction of the ozonised atmosphere, it lived for thirty-five or forty minutes. The number of respirations per minute became smaller, and the animal died in severe general convulsions. The blood, when examined quickly after death, has been found venous in all parts of the body. In both experiments, the temperature of the body was found to be much reduced.

As the reduced temperature of the body in these experiments might have been owing to the current of gas passing quickly over the bodies of the animals, two experiments were made, in which the glass air-chamber was immersed in a water-bath kept at a temperature of 30° C. The animals were supplied with atmosphere at the rate of 13 cubic inches per minute. The general results were the same as in the experiments made without the water-bath, but the temperature of the body on death was still below the normal.

Various experiments were also made on rabbits, with the same general results as in the case of mice. There was evident irritation of the eyes, causing closure of the lids, and the exudation from between their margins of a whitish fluid, probably lachrymal secretion. The respirations were reduced in number from 100 or 110 to from 36 to 30 per minute. In one experiment, only the head of the rabbit was introduced into a glass vessel, into which the stream of ozonised oxygen was transmitted so as to allow the experimenter to count by touch the number per minute of the pulsations of the heart. The result was, that immediately on the introduction of ozone the number of pulsations was much diminished. and the force of the contractions of the heart was so enfeebled that it could not be felt through the wall of the thorax. Still, in the bodies of rabbits killed in an atmosphere of ozonised air, or of ozonised oxygen, the heart was found pulsating, and, as in the other cases, engorged with venous blood.

On breathing an atmosphere of ozonised oxygen ourselves, the chief effects observed were a suffocating feeling in the chest, a tendency to breathe slowly, an irritation of the back of the throat and of the glottis, and a tingling sensation, referred to the skin of the face and the conjunctivæ. The pulse became feebler. After breathing it as long as it was judicious to do, say for five or eight minutes, the suffocating feeling became stronger, and we were obliged to desist. The experiment was followed by violent irritating cough and sneezing, and for five or six hours thereafter by a sensation of rawness in the throat and air-passages.

The action of ozone on several of the chief physiological systems, and on various tissues, was also examined.

1. On the Circulation.—By a suitable apparatus, a frog was imprisoned in a chamber through which a stream of ozonised air, or of ozonised oxygen, passed, while at the same time the web was so placed under a microscope that the circulation in the smaller vessels and capillaries could be readily observed. The result was negative, inasmuch as no appreciable acceleration or retardation of the current of the circulation was seen.

2. On the Reflex Action of the Spinal Cord. - This function was not affected to any appreciable degree.

3. On Muscular Contractility.—By means of a myographion, the work done by the gastrocnemii of frogs, subjected to the action of ozone, was noted. The muscles were stimulated by a single opening or closing induction shock produced by Du Bois Reymond's apparatus and a Daniell's cell. The result was that the contractility and work-power of the muscle were found unaffected, as far as could be appreciated.

4. On the Blood.—When a thin layer of human blood on a slide is exposed to the action of ozone, the coloured corpuscles become paler, lose their definite outline, and if exposed for a period of five or ten minutes to the action of the current, they are dissolved, and a mass of molecular material is seen. The coloured corpuscles of the frog show, after the action of ozone, the formation of a nucleus. By prolonged exposure many of the nuclei apparently pass out of the substance of the corpuscle, numerous free nuclei are seen, and some in the act of separating from the corpuscle have been observed. The colourless corpuscles are contracted into globular masses after the action of ozone. The general effects resemble those produced by a weak acid, such as very dilute acetic acid or a stream of carbonic acid.

5. On Ciliary Motion.—When the cilia of the common mussel (Mytilus edulis) were exposed to the action of ozone, while bathed in the fluid contained in the shell (sea-water), no effect was observed. This is owing to the protection to the cilia afforded by the water.

If a very small amount of water covered the cilia, their action was at once arrested.

From the preceding experiments the following general facts may be stated :---

1. The inhalation of an atmosphere highly charged with ozone diminishes the number of respirations per minute.

2. The pulsations of the heart are reduced in strength, and this organ is found beating feebly after the death of the animal.

3. The blood is always found in a venous condition in all parts of the body, both in cases of death in an atmosphere of ozonised air and of ozonised oxygen.

4. Ozone exercises a destructive action on the living animal tissues if brought into immediate contact with them; but it does not affect them so readily if they are covered by a layer of fluid.

5 Ozone acts as an irritant to the mucous membrane of the nostrils and air-passages, as all observers have previously remarked.

At the present state of this inquiry, it would be premature to generalise regarding the relation between physiological action and the chemical properties of ozone; but we can hardly avoid pointing out that oxygen in this altered condition $(O_2 = 24)$ is slightly denser than carbonic acid $(CO_{2} = 22)$, and that, although the chemical activity of the substance is much increased, yet when inhaled into the lungs, it must retard greatly the rate of diffusion of carbonic acid from the blood, which accounts for the venous character of that fluid after death. If, however, the physiological effect of ozone on respiration were merely due to its greater density, then we would expect its behaviour to be analogous to that of an atmosphere highly charged with carbonic acid. This has been found to be the case, more especially as regards the diminished number of respirations per minute, and the appearance of the blood after death. If, however, this analogy were perfect, we would anticipate that the action of oxygen, partially ozonised, would not have produced death, as the amount of ozone in these experiments certainly did not exceed 10 per cent. As it was, all we have observed is that the animal only lives a somewhat longer time in ozonised oxygen than in ozonised air. We are thus induced to regard ozone as having some specific action on the blood, or in the reflex nervous arrangements of respiration, that future experiments may elucidate.



